

Published in Journal of Agricultural & Food Chemistry, May-June 1982, Pages 486-488, by the American Chemical Society

Isolation and Identification of Additional Beef Flavor Precursors

David M. Alabran

A beef fraction previously obtained by water extraction of lipid-free freeze-dried beef, ultrafiltration of the aqueous extract, and gel permeation chromatography of the ultrafiltrate was further fractionated by high-pressure liquid chromatography, and the components were identified by nuclear magnetic resonance and infrared spectroscopy. The compounds isolated and identified are glutamic acid, lactic acid, phosphoethanolamine, glycerol, creatine, and creatinine and represent 94% of the fraction. Some minor constituent amino acids were also identified.

The correlation of chemical composition with the flavor of beef remains elusive. Some flavor chemistry can be relatively simple, and perhaps the green bell pepper has become a classic example (Buttery et al., 1969), but most flavors are more complex. A significant complication in the case of beef are the modifications produced by cooking. Important nonvolatile flavor components are produced with heating (Tonsbeek et al., 1969), often with large flavor contribution (Tonsbeek et al., 1971). The array of volatiles with cooking is even more complex (Hirai et al., 1973; Mussinan et al., 1973; Wilson et al., 1973) and is not significantly simplified by different cooking conditions (MacLeod and Coppock, 1976, 1977) or by the study of model systems (Boeleus et al., 1974; Qvist and von Sydow, 1974). Since desirable meat flavor is developed by cooking, and most of the large number of aroma constituents thus produced probably play a significant role in the flavor, attempts to simplify the research lead to the study of precursors in the raw meat.

Lipids are important, both directly and as flavor precursors (Forss, 1969; Wasserman, 1972), but previous work at this laboratory was involved with water-soluble beef components. In addition to the amino acids, carbohydrates, and organic acids reported by Jarboe and Mabrouk (1974) in an aqueous beef extract, and their contributions

to beef flavor directly and as precursors, as described, important precursor activity was presented for some gel permeation chromatography fractions of an aqueous beef diffusate (Mabrouk et al., 1969). The determination of the specific chemical composition of one of the more important of these fractions is the effort of this paper. Any beef fraction demonstrating precursor activity warrants analysis in order to ensure the successful formulation of synthetic mixtures, and this addition to the growing list of precursor compounds should assist toward this objective.

EXPERIMENTAL SECTION

The gel permeation chromatography fraction having desirable aroma characteristics was prepared according to the methods described (Mabrouk et al., 1969; Mabrouk, 1973). Briefly, raw semimembranous beef muscle was blended with water, lyophilized, and extracted with petroleum ether. The lipid-free meat was then thoroughly extracted with water, and the aqueous extract was dialyzed [or ultrafiltered: Mabrouk (1973)]. The diffusate was then chromatographed on Sephadex G-25 fine and appropriate aroma characteristics were described. The second fraction thus obtained, averaging 67% of the diffusate, is the subject of this study.

High-Pressure Liquid Chromatography (HPLC). The beef flavor precursor (BFP) mixture obtained in this manner by Sephadex gel permeation chromatography was separated by using a Waters Associates ALC-100 chromatograph equipped with a Model 6000 solvent delivery

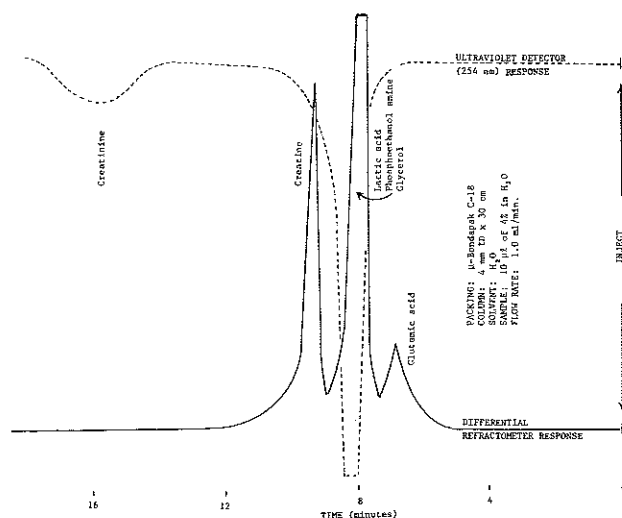


Figure 1. HPLC of the BFP mixture.

system, a differential refractometer detector (sensitivity 1×10^{-7} refractive index units), an ultraviolet detector (254 nm), a Model U6K Universal Injector, and a reverse-phase "micro-Bondapak C-18", 30 cm \times 4 mm i.d. stainless steel column (Waters Associates, Milford, MA). Chromatography was performed at ambient temperature (ca. 25 °C) by using water filtered through a 0.22- μ m Millipore filter before use. Individual components in the chromatogram were manually collected at the outlet of the terminal detector, and several runs were made in order to obtain enough of each component for subsequent identification.

Spectral Identification. Individual compounds were provisionally identified by nuclear magnetic resonance (Varian Associates HA-100 NMR, Palo Alto, CA) and infrared (Perkin-Elmer 267 grating IR, Norwalk, CT) spectroscopy. Confirmation was by chromatographic and spectral comparison with commercially available standards. Amino acids were determined on a Beckman 121 analyzer (Palo Alto, CA).

RESULTS AND DISCUSSION

The best HPLC of the BFP mixture was obtained by using water as the eluting solvent. The chromatogram is reproduced in Figure 1.

Several other solvent combinations were attempted, generally water-alcohol mixtures, but the resulting chromatograms were not as useful. During this experimentation, so that solubility problems in the injector of the chromatograph could be prevented, the sample was prepared in the same solvent mixture being tried as the eluting solvent. In some of these solvents, partial crystallization of the BFP mixture was observed. Careful recrystallization from water-ethanol gave a white solid, which, upon chromatography, corresponded to peak 3 in the Figure 1 chromatogram.

Isolation of the other components in the chromatogram in sufficient quantity for spectral identification required repetitive injections and combined collection of the peak of interest. The eluting solvent, water, was then removed by freeze-drying. On some of these runs peak 2 exhibited slight shoulders, indicating that it was not homogeneous. Separation of the components was accomplished by collecting the leading edge, a center cut, and the tailing edge of peak 2 and then rechromatographing these to obtain pure compounds.

The compounds isolated and identified in this manner were, in order of elution, as follows: peak 1, glutamic acid; peak 2, lactic acid, phosphoethanolamine, and glycerol; peak 3, creatine; peak 4, creatinine. The creatinine peak

Table I. Amino Acids in the BFP Mixture

	mg of amino acid/mg of sample	
	unhydrolyzed	hydrolyzed
lysine		0.00097
histidine	0.00715	0.00455
arginine	0.01049	
aspartic		0.00318
threonine	0.01574	0.00276
serine		0.00266
glutamic	0.09758	0.10589
glycine	0.00329	0.00494
alanine	0.01376	0.01457
valine	0.00377	0.00448
isoleucine	0.00247	0.00352
leucine	0.00544	0.00638
total	0.15969	0.15390

did not have a refractometer response, because of either insufficient concentration or refractive index contrast. Because of the methods of isolation, particularly the components of peak 2, no quantitative data for the individual compounds were obtained, but a total recovery of 94% for the entire chromatogram was determined.

The amino acid composition was determined, both before and after hydrolysis (Liu and Chang, 1971), and the results are listed in Table I. Clearly, there are many more compounds present that are represented by the peaks in Figure 1. They may have been retained on the HPLC column longer than creatinine, buried under the larger peaks, or present in such small amounts as to be imperceptible from the base line. At least in those portions of the chromatogram collected for subsequent spectral identification, there was insufficient amino acid contamination to interfere, or even be noticed, in the NMR and IR spectra.

The total of unhydrolyzed amino acids in Table I is 0.15969 mg, or approximately 16% of the BFP mixture. Of these, glutamic acid was clearly observed and isolated from the Figure 1 chromatogram. Subtracting the amount of glutamic acid from this total leaves 0.06211 mg, or 6%, as the combined other nine amino acids. In the hydrolyzed sample, lysine was observed, arginine was not observed, and the threonine-serine pair was resolved. Again, however, the amino acids other than glutamic represent approximately 5% of the BFP mixture. Additionally, the small differences between the hydrolyzed and unhydrolyzed data seem to preclude the possibility that peptides are no more than trace constituents.

Except for the amino acids, quantitation of the components of the BFP mixture was not possible. However, the components of beef responsible for flavor and aroma need not occur naturally in amounts that are optimum for the desirable organoleptic properties. Efforts at formulating synthetic mixtures, whether of precursors or not, therefore might utilize multidimensional scaling as an indication of flavor success. This procedure constructs a geometrical relationship among sensory data points and a desirable flavor (Alabran et al., 1975; Schiffman, 1974), and such a study is being considered using the compounds reported here and others suggested by the literature.

None of the compounds reported here is unique; nevertheless, the isolated fraction does exhibit precursor activity, just as Mabrouk et al. (1969) report. A complete understanding of beef flavor will probably include synergistic action by compounds which, alone, seem unlikely to make a significant contribution. Isolation and identification of the components of any beef flavor precursor fraction are justified, therefore, and the methods described here seem particularly applicable to aqueous extracts with

precursor activity that are not suitable to other analytical methods.

ACKNOWLEDGMENT

The author expresses appreciation to Dr. Derek Ball, Dr. Frank Bissett, and Leo Holmes for assistance during the research and interpretation of results.

LITERATURE CITED

- Alabran, D. M.; Moskowitz, H. R.; Mabrouk, A. F. *J. Agric. Food Chem.* **1975**, *23*, 229.
- Boeleus, M.; van der Linde, L. M.; de Valois, P. J.; van Dort, H. M.; Takken, H. J. *J. Agric. Food Chem.* **1974**, *22*, 1071.
- Buttery, R. G.; Seifert, R. M.; Lundin, R. E.; Guadagni, D. G.; Ling, L. C. *Chem. Ind. (London)* **1969**, 490.
- Forss, D. A. *J. Agric. Food Chem.* **1969**, *17*, 681.
- Hirai, C.; Herz, K. O.; Pokorny, J.; Chang, S. S. *J. Food Sci.* **1973**, *38*, 393.
- Jarboe, J. K.; Mabrouk, A. F. *J. Agric. Food Chem.* **1974**, *22*, 787.
- Liu, T. Y.; Chang, Y. H. *J. Biol. Chem.* **1971**, *246*, 2842.
- Mabrouk, A. F. *J. Agric. Food Chem.* **1973**, *21*, 942.
- Mabrouk, A. F.; Jarboe, J. K.; O'Connor, E. M. *J. Agric. Food Chem.* **1969**, *17*, 5.
- MacLeod, G.; Coppock, B. M. *J. Agric. Food Chem.* **1976**, *24*, 835.
- MacLeod, G.; Coppock, B. M. *J. Agric. Food Chem.* **1977**, *25*, 113.
- Mussinan, C. J.; Wilson, R. A.; Katz, I. *J. Agric. Food Chem.* **1973**, *21*, 871.
- Qvist, I. H.; von Sydow, E. C. F. *J. Agric. Food Chem.* **1974**, *22*, 1077.
- Schiffman, S. S. *Science (Washington, D.C.)* **1974**, *185*, 112.
- Tonsbeek, C. H. T.; Copier, H.; Plancken, A. J. *J. Agric. Food Chem.* **1971**, *19*, 1014.
- Tonsbeek, C. H. T.; Koenders, E. B.; van der Zijden, A. S. M.; Losekoot, J. A. *J. Agric. Food Chem.* **1969**, *17*, 397.
- Wasserman, A. E. *J. Agric. Food Chem.* **1972**, *20*, 737.
- Wilson, R. A.; Mussinan, C. J.; Katz, I.; Sanderson, A. J. *J. Agric. Food Chem.* **1973**, *21*, 873.

Received for review March 7, 1979. Revised manuscript received November 26, 1980. Accepted January 18, 1982. This paper reports research undertaken at the U.S. Army Natick Research and Development Command and has been assigned No. TP 2024 in the series of papers approved for publication. The findings in this paper are not to be construed as an official Department of the Army position.